

## WHAT IS CLAIMED IS:

1. A method for identifying an inhibitor of cysteine:glucosaminyl inositol ligase, said method comprising:
  - a) contacting a candidate compound with a cysteine:glucosaminyl inositol ligase in the presence of a cysteine and a glucosaminyl inositol or a derivative thereof, under suitable conditions, and
  - b) determining the presence or absence of ligation of the cysteine to the glucosaminyl inositol or derivative thereof ,wherein the substantial absence of the ligation is indicative of a candidate compound that inhibits activity of the ligase.
2. The method of claim 1, wherein the cysteine:glucosaminyl inositol ligase is characterized as having:
  - a) an amino acid sequence with 35% or more sequence identity to SEQ ID NO: 2 or 4, or conservative variations thereof, and
  - b) cysteine:glucosaminyl inositol ligase activity.
3. The method of claim 1, wherein the cysteine is L-cysteine.
4. The method of claim 1, wherein the derivative is D-glucosamine.
5. The method of claim 1, wherein the derivative of glucosaminyl inositol is a fluorescent derivative of glucosaminyl inositol.
6. The method of claim 1, wherein the conditions comprise the presence of ATP.
7. The method of claim 6, wherein the glucosaminyl inositol is 1D-*myo*-inosityl 2-amino-2-deoxy- $\alpha$ -D-glucopyranoside.
8. The method of claim 1, wherein the ligase is produced in an actinomycete.

9. The method of claim 1, wherein the candidate compound is a polypeptide, polynucleotide or small molecule.
10. An inhibitor of cysteine:glucosaminyl inositol ligase identified by the method of claim 1.
11. A method for decreasing the virulence of a pathogenic cysteine:glucosaminyl inositol ligase-producing bacterium in mammalian cells, said method comprising:  
introducing into the bacterium an inhibitor of cysteine:glucosaminyl inositol ligase activity,  
wherein the intracellular presence of the inhibitor decreases activity of the ligase, thereby decreasing mycothiol biosynthesis by the bacterium as compared with untreated control bacterium.
12. The method of claim 11, wherein the cysteine:glucosaminyl inositol ligase is characterized as having:
  - a) an amino acid sequence with 35% or more sequence identity to SEQ ID NO: 2 or 4, or conservative variations thereof, and
  - b) cysteine:glucosaminyl inositol ligase activity.
13. The method of claim 11, wherein the inhibitor inhibits intracellular production of the ligase.
14. The method of claim 11, wherein the inhibitor inhibits intracellular ligase activity of the ligase.
15. The method of claim 11, wherein the introducing comprises culturing the bacterium in the presence of the inhibitor.

16. The method of claim 11, wherein the inhibitor is an anti-sense oligonucleotide complementary to a target region in a messenger RNA that encodes a polypeptide having an amino acid sequence segment with 35% or more sequence identity to the amino acid sequence of SEQ ID NO: 2 or 4.
17. The method of claim 11, wherein the inhibitor is an anti-sense oligonucleotide that hybridizes under intracellular conditions with a messenger RNA that encodes a polypeptide having an N-terminal amino acid sequence as set forth in SEQ ID NO: 2 or 4.
18. The method of claim 11, wherein the bacterium is an actinomycete and the inhibitor inhibits intracellular production of mycothiol.
19. The method of claim 11, wherein the bacterium is an actinomycete.
20. A method for increasing sensitivity of a pathogenic cysteine:glucosaminyl inositol ligase-producing bacterium in mammalian cells to an antibiotic, said method comprising:  
introducing into the bacterium an inhibitor of cysteine:glucosaminyl inositol ligase activity,  
wherein the intracellular presence of the inhibitor decreases activity of the ligase, thereby decreasing mycothiol biosynthesis by the bacterium in said mammalian cells as compared with untreated control bacterium so as to increase sensitivity of the bacterium to an antibiotic.
21. The method of claim 20, wherein the inhibitor inhibits intracellular production of the ligase.
22. The method of claim 20, wherein the inhibitor inhibits intracellular ligase activity of the ligase.

23. The method of claim 20, wherein the cysteine:glucosaminyl inositol ligase is characterized as having:
- a) an amino acid sequence with 35% or more sequence identity to SEQ ID NO: 2 or 4, or conservative variations thereof, and
  - b) cysteine:glucosaminyl inositol ligase activity.
24. The method of claim 20, wherein the introducing comprises culturing the bacterium in the presence of the inhibitor.
25. The method of claim 20, wherein the inhibitor is an anti-sense oligonucleotide complementary to a target region in a messenger RNA that encodes a polypeptide having an amino acid sequence segment with 35% or more sequence identity to the amino acid sequence of SEQ ID NO: 2 or 4.
26. The method of claim 20, wherein the inhibitor is an anti-sense oligonucleotide that hybridizes under intracellular conditions with a messenger RNA that encodes a polypeptide having an N-terminal amino acid sequence as set forth in SEQ ID NO: 2 or 4.
27. The method of claim 20, wherein the bacterium is an actinomycete.
28. The method of claim 20, wherein the bacterium is an actinomycete and the inhibitor inhibits intracellular production of mycothiol.
29. A method for inhibiting growth of a Cys-GlcN-Ins-producing bacterium in a mammal, said method comprising administering to the mammal an effective amount of an inhibitor of intracellular cysteine:glucosaminyl inositol ligase, thereby inhibiting growth of the bacterium in the mammal.
30. The method of claim 29, wherein the bacterium is a mycothiol-producing bacterium.

31. The method of claim 29, wherein the bacterium is an actinomycete.
32. The method of claim 29, wherein the bacterium is an actinomycete and the inhibitor inhibits intracellular production of mycothiol.
33. The method of claim 29, wherein the cysteine:glucosaminyl inositol ligase is characterized as having:
- a) an amino acid sequence with 35% or more sequence identity to SEQ ID NO: 2 or 4, or conservative variations thereof, and
  - b) cysteine:glucosaminyl inositol ligase activity.
34. An inhibitor of cysteine:glucosaminyl inositol ligase, wherein the inhibitor is derived from L-cysteine by replacing the carboxyl group with a moiety that binds the enzyme active site.
35. The inhibitor of claim 34, wherein the moiety replacing the carboxyl group is  $\text{CH}_2\text{OPO}(\text{OH})\text{OR}$ , wherein R is derived from AMP or a cyclitol bearing alkyl residues.
36. The inhibitor of claim 34, wherein the moiety replacing the carboxyl group is  $\text{CONHSO}_2\text{OR}$ , wherein R is derived from AMP or a cyclitol bearing alkyl residues.
37. A method for identifying an inhibitor of acetyl-CoA:cysteinyl glucosaminyl inositol (acetyl-CoA:Cys-GlcN-Ins) acetyltransferase, said method comprising:
- a) contacting a candidate compound with an acetyl-CoA:Cys-GlcN-Ins acetyltransferase in the presence of a cysteine glucosaminyl inositol (Cys-GlcN-Ins) and acetyl-CoA, under suitable conditions, and
  - b) determining the presence or absence of a transfer of acetyl to the Cys-GlcN-Ins, wherein the substantial absence of a transfer of acetyl is indicative of a candidate compound that inhibits activity of the acetyltransferase.

38. The method of claim 37, wherein the acetyl-CoA:Cys-GlcN-Ins acetyltransferase is characterized as having:
- a) an amino acid sequence with 35% or more sequence identity to SEQ ID NO: 14 or 15, or conservative variations thereof, and
  - b) acetyl-CoA:Cys-GlcN-Ins acetyltransferase activity.
39. The method of claim 37, wherein the Cys-GlcN-Ins is 1D-*myo*-inosityl 2-L-cysteinylamido-2-deoxy- $\alpha$ -D-glucopyranoside.
40. The method of claim 37, wherein the acetyltransferase is produced in an actinomycete.
41. The method of claim 37, wherein the candidate compound is a polypeptide, polynucleotide or small molecule.
42. An inhibitor of acetyl-CoA:Cys-GlcN-Ins acetyltransferase identified by the method of claim 37.
43. A method for decreasing the virulence of a pathogenic acetyl-CoA:Cys-GlcN-Ins acetyltransferase-producing bacterium in mammalian cells, said method comprising:
- introducing into the bacterium an inhibitor of acetyl-CoA:Cys-GlcN-Ins acetyltransferase activity,
  - wherein the intracellular presence of the inhibitor decreases activity of the acetyltransferase, thereby decreasing mycothiol biosynthesis by the bacterium as compared with untreated control bacterium.
44. The method of claim 43, wherein the acetyl-CoA:Cys-GlcN-Ins acetyltransferase is characterized as having:
- a) an amino acid sequence with 35% or more sequence identity to SEQ ID NO: 14 or 15, or conservative variations thereof, and
  - b) acetyl-CoA:Cys-GlcN-Ins acetyltransferase activity.

45. The method of claim 43, wherein the inhibitor inhibits intracellular production of the acetyltransferase.
46. The method of claim 43, wherein the inhibitor inhibits intracellular activity of the acetyltransferase.
47. The method of claim 43, wherein the introducing comprises culturing the bacterium in the presence of the inhibitor.
48. The method of claim 43, wherein the inhibitor is an anti-sense oligonucleotide complementary to a target region in a messenger RNA that encodes a polypeptide having an amino acid sequence segment with 35% or more sequence identity to the amino acid sequence of SEQ ID NO: 14 or 15.
49. The method of claim 43, wherein the inhibitor is an anti-sense oligonucleotide that hybridizes under intracellular conditions with a messenger RNA that encodes a polypeptide having 35% or more sequence identity to the amino acid sequence as set forth in SEQ ID NO: 14 or 15.
50. The method of claim 43, wherein the bacterium is an actinomycete.
51. The method of claim 43, wherein the bacterium is an actinomycete and the inhibitor inhibits intracellular production of mycothiol.

52. A method for increasing sensitivity of a pathogenic acetyl-CoA:Cys-GlcN-Ins acetyltransferase-producing bacterium in mammalian cells to an antibiotic, said method comprising:
- introducing into the bacterium an inhibitor of endogenous bacterial acetyltransferase activity,
  - wherein the intracellular presence of the inhibitor decreases activity of the acetyltransferase, thereby decreasing mycothiol biosynthesis by the bacterium in said mammalian cells as compared with untreated control bacterium so as to increase sensitivity of the bacterium to an antibiotic.
53. The method of claim 52, wherein the inhibitor inhibits intracellular production of the acetyltransferase.
54. The method of claim 52, wherein the inhibitor inhibits intracellular activity of the acetyltransferase.
55. The method of claim 52, wherein the acetyl-CoA:Cys-GlcN-Ins acetyltransferase is characterized as having:
- a) an amino acid sequence with 35% or more sequence identity to SEQ ID NO: 14 or 15, or conservative variations thereof, and
  - b) acetyl-CoA:Cys-GlcN-Ins acetyltransferase activity.
56. The method of claim 52, wherein the introducing comprises culturing the bacterium in the presence of the inhibitor.
57. The method of claim 52, wherein the inhibitor is an anti-sense oligonucleotide complementary to a target region in a messenger RNA that encodes a polypeptide having an amino acid sequence segment with 35% or more sequence identity to the amino acid sequence of SEQ ID NO: 14 or 15.



58. The method of claim 52, wherein the inhibitor is an anti-sense oligonucleotide that hybridizes under intracellular conditions with a messenger RNA that encodes a polypeptide having 35% or more sequence identity to the amino acid sequence as set forth in SEQ ID NO: 14 or 15.
59. The method of claim 52, wherein the bacterium is an actinomycete.
60. The method of claim 52, wherein the bacterium is an actinomycete and the inhibitor inhibits intracellular production of mycothiol.
61. A method for inhibiting growth of an acetyl-CoA:Cys-GlcN-Ins acetyltransferase-producing bacterium in a mammal, said method comprising administering to the mammal an effective amount of an inhibitor of intracellular acetyl-CoA:Cys-GlcN-Ins acetyltransferase, thereby inhibiting growth of the bacterium in the mammal.
62. The method of claim 61, wherein the bacterium is a mycothiol-producing bacterium.
63. The method of claim 61, wherein the bacterium is an actinomycete.
64. The method of claim 61, wherein the bacterium is an actinomycete and the inhibitor inhibits intracellular production of mycothiol.
65. The method of claim 61, wherein the acetyl-CoA:Cys-GlcN-Ins acetyltransferase is characterized as having:
- a) an amino acid sequence with 35% or more sequence identity to SEQ ID NO: 14 or 15, or conservative variations thereof, and
  - b) acetyl-CoA:Cys-GlcN-Ins acetyltransferase activity.

66. A method for identifying an inhibitor of MshA glycosyltransferase, said method comprising:
- a) contacting a candidate compound with a mycothiol-producing bacterium, under suitable conditions, and
  - b) determining the presence or absence of 1D-*myo*-inosityl 2-acetamido-2-deoxy- $\alpha$ -D-glucopyranoside (GlcNAc-Ins) within the mycothiol-producing bacterium, wherein the substantial absence of GlcNAc-Ins is indicative of a candidate compound that inhibits activity of the glycosyltransferase.
67. The method of claim 66, wherein the MshA glycosyltransferase is characterized as having:
- a) an amino acid sequence with 35% or more sequence identity to SEQ ID NO: 19 or 20, or conservative variations thereof, and
  - b) MshA glycosyltransferase activity.
68. The method of claim 66, wherein the bacterium is an actinomycete.
69. The method of claim 66, wherein the candidate compound is a polypeptide, polynucleotide or small molecule.
70. An inhibitor of MshA glycosyltransferase identified by the method of claim 66.
71. A method for decreasing the virulence of a pathogenic MshA glycosyltransferase-producing bacterium in mammalian cells, said method comprising:
- introducing into the bacterium an inhibitor of MshA glycosyltransferase activity, wherein the intracellular presence of the inhibitor decreases activity of the glycosyltransferase, thereby decreasing mycothiol biosynthesis by the bacterium as compared with untreated control bacterium.

72. The method of claim 71, wherein the MshA glycosyltransferase is characterized as having:
- a) an amino acid sequence with 35% or more sequence identity to SEQ ID NO: 19 or 20, or conservative variations thereof, and
  - b) MshA glycosyltransferase activity.
73. The method of claim 71, wherein the inhibitor inhibits intracellular production of the glycosyltransferase.
74. The method of claim 71, wherein the inhibitor inhibits intracellular activity of the glycosyltransferase.
75. The method of claim 71, wherein the introducing comprises culturing the bacterium in the presence of the inhibitor.
76. The method of claim 71, wherein the inhibitor is an anti-sense oligonucleotide complementary to a target region in a messenger RNA that encodes a polypeptide having an amino acid sequence segment with 35% or more sequence identity to the amino acid sequence of SEQ ID NO: 19 or 20.
77. The method of claim 71, wherein the inhibitor is an anti-sense oligonucleotide that hybridizes under intracellular conditions with a messenger RNA that encodes a polypeptide having 35% or more sequence identity to the amino acid sequence as set forth in SEQ ID NO: 19 or 20.
78. The method of claim 71, wherein the bacterium is an actinomycete.
79. The method of claim 71, wherein the bacterium is an actinomycete and the inhibitor inhibits intracellular production of mycothiol.

80. A method for increasing sensitivity of a pathogenic MshA glycosyltransferase-producing bacterium in mammalian cells to an antibiotic, said method comprising:  
introducing into the bacterium an inhibitor of endogenous bacterial glycosyltransferase activity,  
wherein the intracellular presence of the inhibitor decreases activity of the glycosyltransferase, thereby decreasing mycothiol biosynthesis by the bacterium in said mammalian cells as compared with untreated control bacterium so as to increase sensitivity of the bacterium to an antibiotic.
81. The method of claim 80, wherein the inhibitor inhibits intracellular production of the glycosyltransferase.
82. The method of claim 80, wherein the inhibitor inhibits intracellular activity of the glycosyltransferase.
83. The method of claim 80, wherein the MshA glycosyltransferase is characterized as having:  
a) an amino acid sequence with 35% or more sequence identity to the amino acid sequence or more sequence identity to SEQ ID NO: 19 or 20, or conservative variations thereof, and  
b) MshA glycosyltransferase activity.
84. The method of claim 80, wherein the introducing comprises culturing the bacterium in the presence of the inhibitor.
85. The method of claim 80, wherein the inhibitor is an anti-sense oligonucleotide complementary to a target region in a messenger RNA that encodes a polypeptide having an amino acid sequence segment with 35% or more sequence identity to the amino acid sequence or more sequence identity to the amino acid sequence of SEQ ID NO: 19 or 20.

86. The method of claim 80, wherein the inhibitor is an anti-sense oligonucleotide that hybridizes under intracellular conditions with a messenger RNA that encodes a polypeptide having 35% or more sequence identity to the amino acid sequence as set forth in SEQ ID NO: 19 or 20.

87. The method of claim 80, wherein the bacterium is an actinomycete.

88. The method of claim 80, wherein the bacterium is an actinomycete and the inhibitor inhibits intracellular production of mycothiol.

89. A method for inhibiting growth of a GlcNAc-Ins-producing bacterium in a mammal, said method comprising administering to the mammal an effective amount of an inhibitor of intracellular MshA glycosyltransferase, thereby inhibiting growth of the bacterium in the mammal.

90. The method of claim 89, wherein the bacterium is a mycothiol-producing bacterium.

91. The method of claim 89, wherein the bacterium is an actinomycete.

92. The method of claim 89, wherein the bacterium is an actinomycete and the inhibitor inhibits intracellular production of mycothiol.

93. The method of claim 89, wherein the MshA glycosyltransferase is characterized as having:

- a) an amino acid sequence with 35% or more sequence identity to SEQ ID NO: 19 or 20, or conservative variations thereof, and
- b) MshA glycosyltransferase activity.

94. A method for identifying an inhibitor of mycothiol biosynthesis, said method comprising:
- a) contacting a candidate compound for inhibition of MshC, MshD or MshA with a mycothiol-producing bacterium, under suitable conditions, and
  - b) determining the presence or absence of mycothiol within the mycothiol-producing bacterium,
- wherein the substantial absence of mycothiol is indicative of a candidate compound that inhibits MshC, MshD or MshA and thereby inhibits mycothiol biosynthesis.
95. The method of claim 94, wherein the bacterium is an actinomycete.
96. The method of claim 94, wherein the candidate compound is a polypeptide, polynucleotide or small molecule.
97. The method of claim 94, wherein the inhibition of mycothiol biosynthesis is by inhibition of cysteine:glucosaminyl inositol ligase.
98. The method of claim 94, wherein the inhibition of mycothiol biosynthesis is by inhibition of acetyl-CoA:Cys-GlcN-Ins acetyltransferase.
99. The method of claim 94, wherein the inhibition of mycothiol biosynthesis is by inhibition of MshA glycosyltransferase.
100. An inhibitor of mycothiol biosynthesis identified by the method of claim 94.

101. A method for increasing sensitivity of a pathogenic mycothiol-producing bacterium in mammalian cells to an antibiotic, said method comprising:  
introducing into the bacterium an inhibitor of endogenous bacterial mycothiol biosynthesis enzyme, wherein the enzyme is selected from MshC, MshD and MshA,  
wherein the intracellular presence of the inhibitor decreases mycothiol biosynthesis by the bacterium in said mammalian cells as compared with untreated control bacterium so as to increase sensitivity of the bacterium to an antibiotic.
102. The method of claim 101, wherein the inhibitor inhibits cysteine:glucosaminyl inositol ligase activity.
103. The method of claim 101, wherein the inhibitor inhibits acetyl-CoA:Cys-GlcN-Ins acetyltransferase activity.
104. The method of claim 101, wherein the inhibitor inhibits MshA glycosyltransferase activity.
105. The method of claim 101, wherein the introducing comprises culturing the bacterium in the presence of the inhibitor.
106. The method of claim 101, wherein the inhibitor is an anti-sense oligonucleotide complementary to a target region in a messenger RNA that encodes a polypeptide having an amino acid sequence segment with 35% or more sequence identity to the amino acid sequence of SEQ ID NO: 2, 4, 14, 15, 19 or 20.
107. The method of claim 101, wherein the inhibitor is an anti-sense oligonucleotide that hybridizes under intracellular conditions with a messenger RNA that encodes a polypeptide having 35% or more sequence identity to the amino acid sequence as set forth in SEQ ID NO: 2, 4, 14, 15, 19 or 20.
108. The method of claim 101, wherein the bacterium is an actinomycete.

109. The method of claim 101, wherein the bacterium is an actinomycete and the inhibitor inhibits intracellular production of mycothiol.

110. A live mutant actinomycete, whose genome comprises a modification in an endogenous enzyme of mycothiol biosynthesis gene or a combination of mycothiol biosynthesis genes, wherein the disruption diminishes the function of the endogenous enzyme while cell surface proteins and lipids are substantially unaffected, wherein the modification results in the mutant actinomycetes exhibiting transient survival in mammalian white blood cells for an immune response-raising period of time, and wherein the gene is selected from *mshC*, *mshD* and *mshA*.

111. The live mutant actinomycete of claim 110, wherein the period of time is from 1 to 30 days.

112. The live mutant actinomycete of claim 110, wherein the survival of the mutant actinomycetes in mammalian white blood cells does not exceed 30 days.

113. The live mutant actinomycete of claim 110, wherein the mutant is more resistant to isoniazid than an untreated control actinomycete.

114. A purified cysteine:glucosaminyl inositol ligase, characterized as having:  
a) an amino acid sequence with 35% or more sequence identity to SEQ ID NO: 2, or conservative variations thereof, and  
b) cysteine:glucosaminyl inositol ligase activity.

115. The purified ligase of claim 114, wherein the ligase catalyzes ligation of the glucosaminyl inositol moiety or a derivative thereof to cysteine.

116. The purified ligase of claim 114, wherein the ligase comprises an amino acid sequence as set forth in SEQ ID NO: 2.



117. The purified ligase of claim 114, wherein the ligase is encoded by a polynucleotide comprising a nucleic acid sequence as set forth in SEQ ID NO: 1.
118. A purified acetyl-CoA:Cys-GlcN-Ins acetyltransferase, characterized as having:
- a) an amino acid sequence with 35% or more sequence identity to SEQ ID NO: 15, or conservative variations thereof, and
  - b) acetyl-CoA:Cys-GlcN-Ins acetyltransferase activity.
119. The purified acetyltransferase of claim 118, wherein the acetyltransferase acetylates Cys-GlcN-Ins or a derivative thereof by acetyl-CoA.
120. The purified acetyltransferase of claim 118, wherein the acetyltransferase comprises an amino acid sequence as set forth in SEQ ID NO: 15.
121. The purified acetyltransferase of claim 118, wherein the acetyltransferase is encoded by a polynucleotide comprising a nucleic acid sequence as set forth in SEQ ID NO: 48.
122. A purified MshA glycosyltransferase, characterized as having:
- a) an amino acid sequence with 35% or more sequence identity to SEQ ID NO: 19, or conservative variations thereof, and
  - b) MshA glycosyltransferase activity.
123. The purified glycosyltransferase of claim 122, wherein the glycosyltransferase comprises an amino acid sequence as set forth in SEQ ID NO: 19.
124. The purified glycosyltransferase of claim 122, wherein the glycosyltransferase is encoded by a polynucleotide comprising a nucleic acid sequence as set forth in SEQ ID NO: 49.

125. An expression vector comprising polynucleotides of *mshA*, *mshB*, *mshC* and *mshD*, wherein the polynucleotide of *mshA* is SEQ ID NO: 49, the polynucleotide of *mshC* is SEQ ID NO: 1 and the polynucleotide of *mshC* is SEQ ID NO: 48.

126. A method for identifying an inhibitor of cysteine:glucosaminyl inositol ligase, said method comprising:

a) contacting a candidate compound with a cysteine:glucosaminyl inositol ligase in the presence of a cysteine, a glucosaminyl inositol or a derivative thereof and ATP, under suitable conditions, and

b) assaying for the generation of pyrophosphate,

wherein the substantial absence of pyrophosphate is indicative of a candidate compound that inhibits activity of the ligase.

127. The method of claim 126, wherein the assay comprises fluoroscopy in assaying for the generation of pyrophosphate.